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Magnetization transfer ratio recovery in new lesions decreases during adolescence in pediatric-onset multiple sclerosis patients

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ABSTRACT

Children and adolescents diagnosed with multiple sclerosis rarely accrue physical disability early in their disease. This could be explained by greater remyelination in children, a capacity that may be lost in adolescence or early adulthood. Magnetization transfer ratio (MTR) MRI can be used to quantify changes in myelin in MS. We used serial MTR imaging and longitudinal random effects analysis to quantify recovery of MTR in acute lesions and to evaluate MTR changes in normal-appearing tissue in 19 adolescent MS patients. Our objective was to determine whether younger adolescents have a greater capacity for remyelination and whether this decreases as patients approach adulthood. We detected a significant decrease in MTR recovery between ages 16 and 20 years ($p = 0.023$), with older subjects approaching typical recovery levels for adult-onset MS. MTR recovery in acute MS lesions decreases with age in adolescents, suggesting loss of remyelination capacity. This may be related to the conclusion of primary myelination or other developmental factors.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease characterized by the formation of inflammatory demyelinating lesions in the central nervous system. Although MS is normally diagnosed in adults, some patients experience clinical onset before the age of 18 (Banwell et al., 2007).

Focal MS lesions vary in their degree of myelin loss. Histopathological analysis of remyelination in the brain of MS patients is limited, and most specimens have been obtained from older adults with long-standing and often secondary progressive MS. Fully demyelinated lesions are common in these patients (Miller et al., 1996), but partially remyelinated "shadow plaques" are also observed (Patrikios et al., 2006), indicating that the adult brain has some ability to repair myelin. It has been suggested that remyelination capacity decreases with age (Fancy et al., 2010; Franklin et al., 2002) in humans and in animal models of induced CNS demyelination (Hinks and Franklin, 2000; Ruckh et al., 2012; Shen et al., 2008; Sim et al., 2002).

If remyelination capacity decreases with age in adults, we would expect children and adolescents with MS to have an even greater capacity for remyelination, and some clinical features of pediatric-onset MS suggest that this may be true. Children and adolescents usually recover completely from their first attack (Bigi and Banwell, 2012; Ruggieri et al., 2004) and rarely accrue physical disability within the first 10 years of the disease (Renoux et al., 2007).

In order to investigate myelination as a function of age, as well as lesional remyelination capacity *in vivo*, an imaging technique sensitive to changes in myelin content is needed. Suitable methods include myelin water fraction (MWF), derived from multicomponent T2 mapping; restricted proton pool size, derived from quantitative magnetization transfer (qMT) imaging; and magnetization transfer ratio (MTR), which is a semi-quantitative measurement of magnetization exchange between free and bound proton pools. We have used an MTR-based approach in this study because data with the required resolution for lesion-based analyses can be acquired relatively quickly, an important issue for pediatric studies. MTR has been histopathologically validated as a marker for myelin in both humans and animals and has excellent quantitative correlation with the Luxol fast blue stain, which is attracted to lipoproteins in the myelin sheath and is used as a standard stain for myelin (Barkhof et al., 2003; Chen et al., 2007; Deloire-Grassin et al., 2000; Dousset et al., 1992; Pike et al., 2000; Schmierer et al., 2004).

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Changes in MTR over time in new lesions exhibit a typical pattern of sudden decrease at the time of lesion formation, followed by partial recovery. We have developed an MTR imaging-based technique that uses longitudinal scanning to identify focal areas of tissue that experience an acute decrease in MTR (corresponding to demyelination) and quantify subsequent recovery (remyelination) (Brown et al., 2012). These "ΔMTR" lesions are distinct from other lesion types normally identified in MS, such as hyperintensities on T2-weighted or gadolinium contrast enhanced scans; because they are identified on MTR images, they are more specific to demyelination (Brown et al., 2012). The MTR timecourse within these lesions can be modeled and produces a statistically powerful measurement of differences in MTR recovery, indicative of remyelination.

If the capacity for repair of MS lesions is particularly robust when concurrent with ongoing myelin maturation or other developmental factors, we would expect that adolescent MS patients would have a greater capacity to repair lesions in early adolescence than in late adolescence/early adulthood, as primary myelination reaches completion.

We quantify changes in lesional MTR in a cohort of adolescents with MS imaged longitudinally from mid-adolescence to early adulthood. We hypothesize that MS lesion remyelination capacity, measured by MTR recovery, (1) decreases rapidly with age in adolescents with MS, and (2) in older adolescents, reaches a level comparable to that seen in adults with MS.

2. Material and methods

2.1. Subjects

All subjects were selected from a single site (The Hospital for Sick Children, Toronto), and all were imaged on the same 1.5 T MRI scanner. Subjects were selected from two studies: our national study of acquired demyelination in Canadian children (Banwell et al., 2011),and from a serial MRI and neurocognitive study of pediatric MS (Till et al., 2011). Subjects selected from the national Canadian study were imaged from the first attack, at 3, 6 and 12 months, and annually. Subjects enrolled in the MRI and neurocognitive study were enrolled at variable timepoints post-MS onset and were imaged two to four times, 12 months apart.

For selection for the present analysis, subjects were required (1) to have a diagnosis of relapsing–remitting MS according to the McDonald 2005 criteria (Polman et al., 2005) — the 2010 McDonald criteria were not available at the time of diagnosis for many of the patients; (2) to have serial scans with high quality MTR imaging, and for these scans to include at least one scan prior to detection of a new lesion, one scan with a new lesion, and one scan at least 6 months after the scan with the new lesion; (3) to be between 15 and 21 years of age at the time of the MRI scan with new lesions; (4) to have their new lesion scan at least 6 months after their incident attack; (5) to have all scans selected to be more than 30 days from a clinical relapse or any corticosteroid exposure; and (6) to either be free of any immunomodulatory therapy or treated with one of the current disease-modifying therapies (DMTs): glatiramer acetate (GA; Copaxone, Teva Pharmaceutical Industries, Petah Tiqva, Israel), or one of two formulations of interferon β-1a (IFN-R; subcutaneous Rebif, Merck Serono International, Geneva, Switzerland or IFN-A; intramuscular Avonex, Biogen, Cambridge, USA). No patient was treated with interferon-beta 1b so we did not include this in our criteria. Very few pediatric MS patients were exposed to other therapies, such as natalizumab or cyclophosphamide, and any such patients were excluded.

2.2. Imaging

Images were acquired on a single 1.5 T GE TwinSpeed Excite 12.0 scanner (GE Medical Systems, Milwaukee, USA). Sequences obtained included: T1-weighted (T1; sagittal SPGR, $T_R = 22$ ms, $T_E = 8$ ms, $1.5 \times 1 \times 1$ mm, flip angle 30°), T2-weighted and proton density-weighted (T2, PD; dual echo axial FSE, $T_R = 3500$ ms, $T_E = 15, 63$ ms, $1 \times 1 \times 2$ mm), and magnetization transfer images (axial SPGR, $T_R = 27$ ms, $T_E = 4$ ms, $1.5 \times 1.5 \times 1.5$ mm, flip angle 12°) with (MT_{ON}) and without (MT_{OFF}) a magnetization transfer pulse (8 ms Fermi). Voxel sizes are all right/left × anterior/posterior × superior/inferior. An adult male volunteer was also scanned at intervals on the same scanner with the same protocol as a "living phantom." MTR images were calculated for each subject at each timepoint using the formula $MTR = 100 \cdot \frac{MT_{OFF} - MT_{ON}}{MT_{OFF}}$ and normalized using the living phantom data, according to the procedure in Brown et al. (2012). This normalization process maps raw MTR measurements onto a calibrated scale, where normal white matter (WM), as defined by the living phantom, has a value of 1 and normal gray matter (GM) has a value of 0, and reduces inter-scanner variability to below the level of inter-subject variability (Brown et al., 2011), as well as corrects potential longitudinal changes in measured MTR on a single scanner. Although MTR measurements are unitless, we will refer to measurements on this scale as being in normalized MTR units (nMU) for clarity.

2.3. Preprocessing

All images for each subject were co-registered (minctracc, McConnell Brain Imaging Centre, Montreal, Quebec, Canada (Collins et al., 1994)) and probability maps for WM, GM, cerebrospinal fluid (CSF) and T2 lesions were constructed using a Bayesian classifier with the T1, T2 and PD images as input (Francis, 2004). T2 lesion masks were automatically generated from the T2 lesion probability maps, reviewed and, if necessary, edited by trained readers. Longitudinally-consistent high confidence WM and GM masks (HCWM, HCGM) were constructed, consisting of tissue identified as having greater than 85% probability WM or GM at every timepoint. Finally, *de novo* ΔMTR lesion masks were generated as previously described (Brown et al., 2012). ΔMTR lesions were segmented from MTR difference images by identifying focal areas of newly-appearing MTR signal decrease. *De novo* ΔMTR lesions were defined as those occurring in previously normal-appearing WM.

2.4. Analysis

Each lesion was assigned a unique identifier and regions of interest (ROIs) corresponding to each lesion were identified on all scans. MTR values of voxels within the ROI were averaged, producing a mean lesion MTR measurement at each timepoint. Together with the elapsed time relative to the lesion appearance, these data form an MTR timecourse for the lesion, showing MTR evolution from before lesion formation, through acute inflammation, to post-lesion follow-up. From other data (Brown et al., 2012) we have observed that MTR values in lesions change rapidly during the acute inflammatory phase, decreasing from about 1 month before the lesion is observed, recovering partially by 3 months, and then remaining relatively stable in the longer term (Brown et al., 2012). MTR measurements made during the period of acute lesion formation could be influenced by inflammation and its resolution. For these reasons, only samples acquired outside the acute period (defined as 1 month prior to 3 months after lesion formation) were included in our statistical models, although all data have been graphed to illustrate the entire timecourse.

The primary goal of the analysis was to detect changes in the amount of MTR recovery with age. However, disease duration and treatment status might be significant confounding factors. Since disease duration and age are correlated longitudinally, these should not both be factors in the same model. Therefore, we used age at onset in place of disease duration, which corrects for any effects related to disease duration, independent of the age at which a new lesion is observed.